MS. mailed 6-18-69

From crossing-over to developmental genetics

Curt Stern
Departments of Zoology and Genetics
University of California, Berkeley

It has been suggested to me by the conveners of this symposium that a backward glance on some of my activities in genetics would be appropriate. This causes some conflicting reactions. On the one hand it is not usual to single out one's own work but on the other it is fun to reminisce occasionally. To reminisce should not be a purely egotistic procedure. It might serve to show the continuity of genetics during the decades of one individual's experiences. Human generations overlap. Each generation is not just a bridge between the past and the future but actually participates in each of them. Old and young share some of the same experiences. On this basis my remarks are particularly addressed to the younger generation, students and staff members alike, to show how unexpected connections between different experiences appear, how some findings may remain dormant for years and then take on new meanings.

I did not obtain my doctor's degree in genetics. I was a student in my native Germany of the protozoologist and great general biologist Max Hartmann and my thesis dealt with the cytology and a bit of the physiology of a freshwater protozoan. This was in the early twenties. While I was happy with my type of research, my thinking was also under the influence of the rise of genetics. Morgan's Physical Basis of Heredity HAD just appeared in a German translation -- the original American literature of the World War I period was not yet available -- and it, together with Goldschmidt's books and papers on intersexuality, genetic mechanisms and physiological genetics, impressed us as depicting one of

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the most important periods in the history of biology. Since I had not been active in genetics myself, I was greatly astonished to be offered a postdoctoral fellowship to work in the fly room at Columbia University. Morgan, Bridges and Sturtevant represented a holy trinity to me and I have always been grateful for the extraordinary good luck of having been a student and friend of these great men.

My first contact with crossing over occurred while I was still in Germany. I read widely in the literature of Drosophila genetics including the comprehensive three books on the genes of the first, second and third chromosomes. When, after arrival at Columbia University, I told Dr. Morgan of my eager literary studies, he smiled and said that these books were records rather than reading material and that he had not thought that anyone would be so stupid as to read every page of them.

In my reading I came across a paper by Goldschmidt published in 1917 while he was in this country unable to return to blockaded Germany. The paper had appeared in Genetics, in German: "Crossing over ohne Chiasmatypie". It was an imaginative hypothesis in which crossing over was supposed to be the consequence of the genes leaving and rejoining a nongenic chromosomal skeleton rather than a consequence of breakage and reunion of chromosomal segments. Goldschmidt's suggestion was criticized by a note of Sturtevant's which bore the title "Crossing over without chiasmatype?", the questionmark standing for an emphatic: "No". I myself formulated some arguments against Goldschmidt's hypothesis which I put in the form of a little manuscript which I handed with great trepidation to Professor Goldschmidt in whose department I then held a minor position. For half a year I heard nothing about my paper. Then one day Professor Goldschmidt's secretary returned the pages to me. No comment!

I now turn to my work on the so-called cytological proof

of crossing over and the events which led up to it. When I was at Columbia, I studied the effect of age and temperature on crossing over in a region of the X-chromosome which had just become accessible to such study. Sturtevant had discovered a mutant which was located to the right of all other X-linked mutants. This mutant caused the formation of smaller than normal bristles strangely enough in females only. The short-haired females seemed analogous to the then newly fashionable bobbed hair of women and Sturtevant named the mutant "bobbed". In laboratory discussions it came to light that the "non-Drosophilist" Professor Burlingame of Stanford University, during a period when the Morgan group had temporarily moved to Stanford, had made an interesting suggestion. He had wondered whether the normality of males who carry bobbed in their X-chromosomes might be due to the presence of a normal allele of bobbed in the Y chromosome. This turned out to be true when I found a female fly in a bobbed stock which had normal, not bobbed, bristles and was able to show both genetically and cytologically that it carried a Y chromosome in addition to its two X's. Obviously, the wild type female had originated from the process of non-disjunction of the sex chromosomes in either her mother or father. Having established this I might have written a paper about it and proceeded to something else. But, for reasons of habit or for quelling any secret doubts about the validity of my findings, I watched for more normal bristled females in my bobbed stock. Soon I found a second case, analyzed it and confirmed that it also was XXY. Continuing, I found a third. Again it was XXY. Inertia led to a fourth finding. She was not XXY! Her chromosomal make-up was different from both XX and XXY females. She carried one typical X-chromosome and another sex chromosome which consisted of an X and a long arm of the Y chromosome, Y^L, attached to it. The normal phenotype of this "X XY^L" fly showed that it is the long arm of the Y chromosome which carries a normal allele of bobbed.

It was at this stage that I remembered a passage in a

passage in a lengthy protozoological paper which I had read several years earlier. In 1923, Karl Belar had published a beautiful account of meiosis in the unicellular heliozoon Actinophrys sol. This form alternates between mitoses and meiosis and Belar had shown that the intricate processes of chromosome pairing, bouquet formation, pachytene condensation and other meiotic prophase phenomena in this protozoon fully duplicate the meiotic processes which had been the subject of many studies in grasshoppers, flatworms and other organisms, animal and plant. In the discussion of Belar's paper the following sentences occurred, translated from the German: "It would be anachronastic if in this era of Morgan's discoveries a cytological paper .... would not take a stand with respect to the chiasmatype theory. The beautiful diplotene stages actually provoke such a discussion. Unfortunately, however, nothing can be said here either pro or con. And that is true not only for Actinophrys but also for other objects. Study of fixed preparations can lead to a decision only when the two chromosomes of a pair are morphologically distinguishable, i.e. structurally different." Belar had seen that morphologically identical homologous chromosomes cannot result in new types of chromosomes from crossing over but that heteromorphic homologues can do so. It was implicit in Belar's statement that only double heteromorphism could lead to new chromosomes. Crossing over between a pair of homologues different from each other at a single point would result in two chromosomes indistinguishable from the two original ones. If, however, the two homologues differed at two separate parts, for instance at both ends, then crossing over somewhere between the ends could recombine the markers so that two visibly new chromosomes would result. When I had found the Drosophila female who had one normal rod-shaped X-chromosome and one X-chromosome at whose proximal end there was an attachment of the long arm of the Y chromosome, I held in my hand one half of the required chromosome configuration with which to test the theory of crossing over.

The singly heteromorphic pair of X-chromosomes by itself was of no use but it invited a search for another heteromorphism somewhere else along the X-chromosomes. If I could find it I would be in business! But where would I find it? Apart from Mrs. Morgan's attached X-chromosomes and her ring-X and from my XY^L translocation, no microscopically visible chromosome aberrations had yet been observed by anyone.

I remember how I discussed my hopes with Franz Schrader, then at Bryn Mawr, on a visit of his to Columbia University. He told me that he had recognized the situation long ago. In grasshoppers, the distinguished cytologists Wenrich and Carothers had described singly heteromorphic chromosome pairs and he, Schrader, had gone to Wenrich and suggested the crucial experiment: look for heteromorphism at a second site of your chromosomes and then see whether you recover not only the originally different homologues but in addition two new types, resulting from crossing over. But the suggestion did not appeal to Wenrich and nothing had been done.

I tried various ways of combining the few chromosome aberrations known in Drosophila in the hope of obtaining new chromosomes by crossing over, but in vain. Then, in 1928, H. J. Muller made it known that X-rays do not only produce gene mutations as he had shown the year before but that they can break chromosomes and lead to the production of an abundance of chromosome aberrations. If I could only get some of the new chromosomes from Muller, I thought, I might be able to perform "the" experiment. It either did not occur to me to make my own X-ray aberrations or I felt that the task might require experiments too long in duration. In any case, I wondered whether I should write to Muller and ask for his help. I admit that this was a ticklish business for a young man. I had to tell Muller of my plan and ask him whether he himself was planning along similar lines. Should he reply "Yes, this obvious experiment is under way in my lab," then I

would have lost my opportunity. But what else could I do? So I wrote to Muller who was then at the University of Texas and received a most generous reply. He had realized from some work of mine, he wrote to me in Germany, that I was pursuing the problem of a cytological proof of crossing over, that he himself had no similar plans and that he would send me various stocks with chromosome aberrations some of which might suit my purpose. And so he did from 1928 to 1930. Unfortunately, however, none of them was useful to me. The chromosomes did not agree with the labels on the vials. The aberrations had been lost or the analyses had been incomplete. Early in 1931, however, I received a translocation between the X and the fourth chromosome, the now well-known "Bar-Stone" translocation named after Wilson Stone. In essence, it contained an X-chromosome whose distal half had been removed so that it is a short chromosome. If my XY^L chromosome could be called "long X with long Y^L" then the Bar-Stone translocation was "short X, without Y^L". I was in business. The work was done within a few months and the paper dedicated to Professor Morgan on his sixty-fifth birthday. He wrote me a friendly letter of thanks saying that he was "glad that at last we have some objective evidence upon which to rest the [crossover] theory." Looking back, however, I must agree with the evaluation given by Dunn in his Short History of Genetics: "So thorough had been the genetical experiments, that Stern's demonstration seemed anticlimactic."

In the context of reminiscences as well as for the benefit of sociologists of science who perhaps may find food for their thoughts, let me recount some aspects of my first report on the cytological proof of crossing over. By the summer of 1931 I had completed the work, had written the paper which was accepted for publication and had then gone on vacation. At the end of this p eriod I went to Munich to attend the September meeting of the German Genetics Society and to present my results. With me came my fiancee who on the day of my speech

presented me with a set of beautifully arranged attached and translocated candy bars. I gave my paper with the enthusiasm of a successful youth. Soon after, one of my colleagues from the Kaiser Wilhelm Institut came to me and said: "I didn't want to spoil your fun but while you were on vacation a paper came out written by Harriet Creighton and Barbara McClintock who did experiments in maize equivalent to what you just announced as unique." May I confess that I am still grateful to my colleague for permitting me the feeling of triumph for half an hour longer than I would have had it if he had told me about the Creighton-McClintock paper before my talk.

You are aware that the two reports on the cytological proof of crossing over, and a few subsequent corroborations, were for a long time regarded as evidence for a breakage-reunion mechanism of crossing over. And you are aware that this was an unfounded belief. Copy-choice as first suggested by Belling could also account for the production of cytologically new chromosomes from doubly heteromorphic pairs. It was not until 30 years later that the breakage-reunion theory was proven, by the use of doubly labelled prokaryotic chromosomes, those of the lambda bacteriophage. In eukaryotic organisms such as Drosophila and Zea mays a direct proof of breakage-reunion is still not available.

While the 1931 papers were convincing to most investigators there was one prominent exception. Hans Winkler had just published his book on the theory of gene conversion. This term which now has a different meaning from that attributed to it by Winkler was the basis of his novel theory of crossing over. Winkler did not believe in chromosome exchange but postulated that frequently genes change spontaneously during meiosis from one allele to another. If, for instance, a chromosome carries the genes A and B and its homologue the alleles a and b then conversion of A into a, and of a into A would create chromosomes of the types aB and Ab. They would

be genetic crossover chromosomes but cytologically unchanged chromosomes. I had been involved in a controversy with Winkler about his theory. I published a lengthy review and attempted repudiation of his book, he reviewed my review, and I reviewed his review. When I had obtained the new chromosomes from the doubly heteromorphic ones I felt that the case had been decided against the theory of conversion. But not so Winkler. In essence, he reacted as follows. "If you have two homologous chromosomes, one with and the other without a translocated piece you must assume that a pair of alleles is involved at the translocation site, K leading to attachment of the translocated piece and k to its detachment. Gene conversion will change K into k, and vice versa resulting in reciprocal detachment and attachment. If you have a long rod chromosome with C for continuity of the chromosome at a specific site and if C converts itself to c the long rod will separate at the c site into two shorter segments. And if you have two chromosome pieces with c for separateness and if c converts itself to C the two pieces will zip together to form a single long rod." Perhaps, this reasoning is not too convincing, but you must admit its ingenuity.

Let me go back in time to 1925. In that year Bridges discovered a strange effect of the dominant X-linked gene for fine bristles and slow development, Minute-n. He dealt with females in one of whose X-chromosomes there was the dominant gene for not-yellow as well as Minute-n and in whose other X-chromosome were present the recessive allele for yellow and that for not-Minute. Such flies are non-yellow and Minute. Unexpectedly, however, many of them had somewhere an area of yellow not-Minute phenotype. From his analysis of numerous such "spots" on females of the stated or of related genotypes, Bridges concluded that Minute-n had the property of sometimes eliminating the chromosome on which it was located thus resulting in spots in which only the X-chromosome occupied by yellow and not-Minute was left. Such losses of an X-chromosome were not unknown. They accounted

for the origin of many gynanders which usually are flies composed of a mixture of large female and male areas. Elimination of an X-chromosome had occurred during early cleavage, resulting in equal or similar numbers of XX and X nuclei. The new feature of Bridges' spot mosaics was the apparent late developmental origin of the new genotype as well as the specific influence of Minute-n on the postulated elimination of an X-chromosome.

Not long after the publication of Bridges' stimulating paper I found that autosomal Minute genotypes also lead to the appearance of aberrant spots. They could be explained in terms of loss of autosomal genes. However, it appeared that not a whole autosome was lost but only one or the other of its two long arms. Soon another fact became apparent. Females who carried not-yellow, Minute-n and not-bobbed in one of their X-chromosomes and yellow, not-Minute, bobbed in the other formed yellow not-Minute spots as had been shown by Bridges. However, instead of being of bobbed phenotype the bristles were normal. Had the whole Minute-n-carrying X-chromosome been eliminated the genotype of the spots should have been yellow not-Minute bobbed. Why then did bobbed not appear phenotypically?

One possible explanation was that the effect of bobbed was non-autonomous: it did not produce its phenotype if present in a small area of a not-bobbed fly. There was a precedent for the assumption of non-autonomy. Most genes of Drosophila were known to act autonomously in mosaics but Sturtevant's demonstration of non-autonomy of the vermilion gene was a famous exception. There was an alternative explanation of the not-bobbed phenotype of the spots. Could it be that Minute-n did not lead to the elimination of a whole X-chromosome but only of part of it, retaining in the cell nucleus the proximal section with the not-bobbed allele?

The hypothesis of only partial elimination of the X-chromosome could be tested by means of Muller's Theta-duplication.

This was a short section of the X-chromosome, containing the not-yellow allele, which was attached to the very small short arm of an X-chromosome. Females who had Theta attached to the Minute-n carrying X-chromosome, and possessed yellow in the homologous X-chromosome, were not yellow. If their whole Minute-n carrying X-chromosome was eliminated, including the Theta attachment, then the resulting spot would be yellow in phenotype. If, however, part of the X-chromosome including Theta was retained then the phenotype of a spot would be not-yellow. If was the latter situation which was observed and it agreed with Patterson's prior finding that in spots induced by X-rays "not the whole X-chromosome was eliminated".

Why and how did Minute-n and the autosomal Minutes lead to partial loss of chromosomes? This puzzle led to a variety of experiments to find a way of solving it. Ultimately the answer was that actually no partial loss occurred at all. The decisive experiments on which I stumbled involved the finding that spots for X-linked genes occurred not only in the presence of X-linked Minutes but also in that of autosomal ones. In a given experiment one X-chromosome carried the recessive yellow and the dominant non-singed genes, the other not-yellow and singed. Among 15 spots 2 were yellow and not-singed, 2 others not-yellow singed and 11 were twin spots consisting of a yellow not-singed area adjacent to a not-yellow singed. How was all this possible? In another experiment one X-chromosome contained both recessives yellow and singed, the other both normal alleles. Here, among 160 spots, 110 were yellow and singed, 43 yellow not-singed and 7 not-yellow singed. How to account for these results? It turned out that the overall solution was based on the unexpected existence of "somatic crossing over", not on chromosomal loss. A very lengthy paper provided the evidence, "in Minute detail", as Dr. Patterson teasingly characterized it.

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two then predominant great branches of biology, genetics and experimental embryology, as represented by Thomas Hunt Morgan and Hans Spemann. It was one of my goals to contribute to a fusion of the two fields which had developed largely independently of each other. An opportunity offered itself when I made use of the Theta duplication in the analysis of somatic crossing over. I observed that Theta led to the presence in a specific region on the thorax of Drosophila melanogaster of a bristle that is not present in non-Theta flies. This "interalar" bristle is a normal feature of related dipteran species. By means of somatic crossing over I obtained mosaics for Theta/not-Theta and interpreted the findings in terms of induction of interalar bristle formation in the epidermis by the underlying tissue. I wrote a manuscript and sent it to Dr. Sturtevant in the hope that he would introduce it to the Proceedings of the National Academy of Sciences. He returned the manuscript together with a letter to the editor of the Proceedings submitting the paper for publication. But there was a second letter, addressed to me, in which doubts were expressed concerning the validity of my Spemannian interpretation. The result was that the manuscript remained a manuscript: unpublished. Using the then recently invented method of Beadle and Ephrussi, I turned to transplantation of testes within and between different species of Drosophila and succeeded in introducing the concept of induction in the determination of testes shape. Different genotypes cause different shapes by way of different growth inducers.

In 1941, one of my graduate students, Adair Brasted, published her doctoral thesis: "An analysis of the expression of the mutant 'engrailed' in Drosophila melanogaster". Engrailed is a mutant with multiple effects, the most interesting one of which is the formation of a secondary, mirror image sex comb on the male foreleg in addition to the single primary sex comb ~~on the male foreleg in addition to the single primary sex comb~~ of normal males. The Discussion attempted to

interpret the appearance of sex combs in males and their absence in females by making use of the embryological field concept. It led to the following statement concerning gynanders:

"If a sex-comb should appear in a region composed of female tissue but surrounded by male tissue, then it might be said that a sex-comb field was present and sex-comb formation persisted in spite of the female constitution of the responding tissue. A search for such material has thus far revealed no crucial case."

What was needed, then, were numerous gynanders in the hope that some of them would be sex mosaics in the critical region. Gynanders are rare and few were found until, five years later, Griffin and Lindsley in an abstract announced the existence of an unstable ring X-chromosome whose frequent elimination represented a tool for gynander production. The unstable ring was made available to us and soon afterward Dr. Aloha Hannah and I accumulated many gynanders including some of female/male mixtures in the sex comb region. Their study revealed an unexpected situation. Female tissue even if present in the sex comb forming region of a mostly male tarsus differentiated female bristles only, not sex comb teeth. Conversely male tissue that occurred on a mostly female foreleg at the region which is homologous to that of the sex comb in males, differentiated typical sex comb teeth not female bristles. We concluded that a sex comb field is present in both sexes and that the sexual difference of the forelegs is due to differential response of female and male tissue to an invariant singularity of the region. Later Dr. Chiyoko Tokunaga, by means of somatic crossing over, obtained mosaics for the autosomal mutant engrailed and established that the difference between engrailed and not-engrailed sex comb differentiation lies not in a difference between presence and absence ofa "field for secondary sex comb formation" but in differential response of the two genotypes to an invariant "prepattern singularity". Other

pattern phenotypps such as produced by the gene "achaete" which removes, i.e. does not differentiate, specific bristles at specific sites were also shown to be due to genetically different response of tissues to invariant prepatterns. The Theta duplication that leads to differentiation of the inter-alar bristle also belongs to this class of pattern genotypes. A reanalysis of the mosaics for Theta which had been left ununderstood in the unpublished manuscript referred to earlier now saw the light of public scrutiny in a paper in Roux's Archiv.

For a while it seemed as if all mutants studied were alike in affecting only responses but not prepatterns. Later, indications of prepattern effects of some mutants were obtained and, finally, rather clear evidence for such a mutant was found in the sex comb of $\underline{ey}^D$. This genotype causes the appearance of a multiple sex comb. Mosaics for $\underline{ey}^D$, if in the sex comb region, produce multiple comb sections not only out of $\underline{ey}^D$ but even out of not-$\underline{ey}^D$ tissue. The underlying abnormal differentiation of tarsal segmentation acts as a new prepattern that forces multiple differentiation upon both $\underline{ey}^D$ and not-$\underline{ey}^D$ tissue.

My story has taken you from meiotic to mitotic somatic crossing over as fundamental topics worthy of analysis and then to the application of somatic crossing over as a tool in the study of developmental genetics. Our interest in the latter area is still lively but I have recently returned to my old love, crossing over $\underline{per\ se}$. It is known from the work of various authors that meiotic crossing over can take place within a gene and it occurred to me to wonder whether in Drosophila somatic crossing over too could be intragenic. A suitable genetic material for answering this question is given by the white locus. Meiotically, Green and Judd have separated the sites of different white alleles by observing normal red eyed segregants originating from white eyed females. In these cases two different non-complementing white alleles, here designated as w^1 and w^2 in the trans-

configuration $w^1 +^2 / +^1 w^2$, may give rise by meiotic crossing over to $+^1 +^2$ normal gametes. Could somatic crossing over accomplish the same?

Professor Morgan once explained to a visitor that he had a series of experiments under way, some reasonable, some slightly foolish and some so foolish that he would not talk about them. In a way I felt that the attempt to observe the results of intragenic somatic crossing over belonged in the last of Morgan's categories. How small would be the chance to discover such an event, if it occurred at all! But he who does not dare may never win. There was an element involved which might help to yield the impossible. An eye of Drosophila is compounded of many facets, about 750. Two eyes amount to 1500 facets and a thousand flies to a million and a half. If during development of a fly with the non complementing, i.e. white-eyed, constitution $w^1 +^2 / +^1 w^2$ somatic crossing over between the w^1 and w^2 sites had created a normal $+^1 +^2$ chromosome, a pigmented spot would be produced. I looked at a paltry six thousand flies. They corresponded to about nine million mitotic events (or more depending on whether all or only some of the pigment cells of a facet are sufficient to give rise to an observable spot). In four of the mitotic events intragenic crossing over had occurred, as judged by 4 pigmented spots of from about 2 to 16 facets.

One can use this result for making an estimate, however rough, of the total frequency of somatic crossing over during the development of Drosophila. The meiotic map length of the white cistron between the sites w^1 and w^2 is about 0.0146 per cent and the sum of the map length of all chromosomes is about 280. This makes the total map length 2×10^4 times longer than the white section. If -- and this is a very inaccurate "if" -- the mean freqeency of crossing over anywhere is like that observed in the small w^1-w^2 sample,

then the frequency of cells with a cross over is of the order of one, or one tenth, per cent. Neither of these two values is a negligible one from the point of view of students who are looking for possible somatic crossing over in tissue cultures or elsewhere.

Here my story ends. It is not exhaustive. In decades of activity many different lines are followed, some for short, others for longer distances. After decades of activity one's part in the growth of science seems unrelated to oneself. Is the person who is alive now really the same who did some work forty years ago?

I talked about some adventures in classical genetics. Is molecular genetics separated from classical genetics by a revolutionary break? I do not think so. DNA was discovered by Miescher in 1869, in the nuclei of pus cells and, later, in the sperm of fish. It was an interesting substance -- but what of its meaning?

It took decades of cytological research, observation and thinking, decades of classical genetics in terms of factor analysis, linkage and recombination to prepare the answer to the meaning of DNA. When the answer came ---from Avery in 1944 -- a great advance had been made, without revolution. Everything remained in place, but the dreams of the classical geneticists of understanding gene structure, gene mutation and gene regulation had begun to come true.

This work was carried out under the auspices of the United States Atomic Energy Commission and supported in part by NSF grant GB-23024.